THE INHIBITORY ACTION OF CERTAIN ANILINE DYES UPON BACTERIAL DEVELOPMENT.[1]

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FIRST COMMUNICATION.

A. Historical Review. While Pfeffer[2] had demonstrated in 1886 that certain aniline dyes are capable of producing a harmful effect upon the higher plants, Rozsahegyi[3] seems to have been the first to point out that a similar effect is exerted upon certain bacteria (1887). His experiments were conducted by embodying the dyes in nutrient gelatin and inoculating the tubes with the different organisms. The number of the dyes as well as of the types of bacteria which were studied is not large (carmine, methylene blue, gentian violet, vesuvin, fuchsin, and methyl violet in reference to the Bacillus cyanogenus, pyocyaneus, cuniculicida, choleræ gallinarum, murisepticus, choleræ Asiaticæ, Finkler-Prior and choleræ suum), but the writer has clearly demonstrated not only that certain organisms do not develop in the presence of certain dyes, but also that certain dyes possess a *selective* action for certain organisms. He has thus pointed out that while the bacillus of rabbit septicemia readily grows in the presence of carmine and vesuvin, and not in the presence of gentian violet, the chicken cholera bacillus does not grow in the presence of vesuvin, but does so without difficulty in the presence of gentian violet. He accordingly suggests that this method may be used to advantage in differentiating similar bacterial species from one another.

Rozsahegyi's experiments very curiously seem to have escaped the attention of a number of subsequent investigators, and Stilling in particular, whose detailed investigations into the bactericidal action of aniline dyes were published in 1890, does not mention them. It appears, however, from the meagre literature upon the subject that the inhibitory effect of certain dyes upon bacteria was known to a number of observers, even though the observation of this fact led to no further investigation. Cornil and Babes[4]

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[1] Received for publication April 15, 1913.

[2] Ueber Aufnahme von Anilinfarben in lebende Zellen. Untersuchungen aus d. botanischen Institut in Tübingen, 1886, ii.

[3] Centralbl. f. Bakt., Orig., 1887, ii, 418.

[4] Les bacteries, 1890, third edition, i, 76.

thus mention in the third edition of their work on bacteria that the movements of certain organisms are arrested when these are placed in aqueous solutions of certain dyes, but they evidently also noted the selective action of their staining solutions, for they remark that the cholera vibrio remains alive even after being stained.

Kremianski,[5] of Charkoff, further advocated the use of pure aniline and of aniline dyes, as inhalations in the treatment of phthisis, upon the basis of their bactericidal power. Incidentally it is interesting to note that a curative action of aniline upon wounds has long been assumed by aniline dye-workers, who are in the habit of dusting abrasions, etc., with dyes, and maintain that the healing process is thereby hastened.

In 1890 there appeared three publications dealing with the bactericidal action of aniline dyes, viz., one by Penzoldt,[6] who records the investigations made under his direction by Beckh, and two others by Stilling.[7] Beckh found that methyl violet, malachite green, and rose Bengale (in aqueous solutions, varying in strength between 2 and 10 per cent.) would kill staphylococci, and that trimethyl rosaniline, phenyl blue, and methylene blue (in 0.2 to 3 per cent. solutions) will inhibit their development, or may even arrest it completely, while (acid?) fuchsin, corallin, eosin, methyl orange, vesuvin, tropeolin, scarlet red, and indulin are without effect. The experiments were carried on by impregnating silk threads with the organisms and then planting them in nutrient gelatin containing the dyes in various concentrations. The same observer notes that concentrated solutions of methyl violet, malachite green, trimethyl rosaniline, and phenyl blue prevent the growth of the anthrax bacillus.

Stilling has been less explicit in reference to the names of the individual dyes which he investigated. In his first publication he remarks that "certain aniline dyes, notably those of violet color, possess all the properties which one can demand from a good antiseptic" (p. 6). In a foot-note (p. 8) he states, "For brevity's sake I shall use the term methyl violet to designate the entire group of violet aniline dyes." In addition to these, still others were tested, however, for he mentions at one place (p. 12) that fuchsin, methylene blue, rhodamin, vesuvin, and many others were examined, but that none of these were as efficacious as the violets. The next best were certain auramins. Ethyl violet he found even more active than "methyl violet," but as this is contaminated with arsenic it could not be used in his animal experiments, nor in his therapeutic investigations. At Stilling's request the firm of Merck & Co. then introduced a mixture of those dyes which he had found to be satisfactory into the trade, and designated the product as pyoctanin. Their blue pyoctanin is essentially a mixture of chemically pure methyl violet 6 B, dahlia, and benzoyl violet (bleu de Paris), while their yellow product is said to be chemically pure auramin.

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[5] Cited by Stilling, second communication, p. 4.

[6] Arch. f. exper. Pathol. u. Pharmak., 1890, xxvi, Heft 5 und 6.

[7] Anilin Farbstoffe als Antiseptica und ihre Anwendung in der Praxis, Mittheilung 1 and 2, Strassburg, 1890, Verlag v. K. J. Trübner.

As regards the manner in which the dyes in question were tested, Stilling and his co-worker, Wortmann, started from the assumption that any effect achieved in the case of the more "resistant" bacteria would warrant the inference that a similar effect might be expected in the case of those "nobler forms," which require special media for their successful cultivation. As I shall have occasion to show later on such a conclusion is totally unwarrantable. So far as Stilling's and Wortmann's work with pure cultures is concerned, they have established the fact that staphylococcus aureus does not develop in dextrose broth containing methyl violet (blue pyoctanin) in dilutions down to 1 to 1,000,000 and even of 1 to 2,000,000 (second communication, p. 21); that the typhoid bacillus succumbs in dilutions of 1 to 6000 in three hours; Bacillus subtilis in dilutions of 1 to 4000 in seventy-five minutes; Bacillus anthracis in dilutions of 1 to 16,000 in ten minutes; subtilis and anthrax spores in dilutions of 1 to 1,000,000 in twenty-four hours. Similar results were obtained with cultures of several moulds, such as penicillium glaucum, phycomyces nitens, and aspergillus niger. The remaining experiments were not conducted with pure cultures nor with other pathogenic organisms, but had reference merely to mixtures of the "common putrefactive organisms." As a result of their findings, Stilling concludes that in sufficient concentration the dyes in question will effectively prevent the development of practically all the different bacteria. Their mode of action Wortman explains as follows: When the organisms are first placed in the colored solutions the dye is deposited in the intermicellary spaces of the covering membrane, which is thereby stained. From these it can be extracted again by merely placing the cell in water. He adds that even without supposing that any chemical union has taken place, the mere occlusion of the intermicellary spaces would suffice to more or less seriously affect the metabolism of the cell and could thus lead to more or less serious consequences without actually causing death. On longer exposure or in the presence of a greater concentration of the dye a certain quantity of the latter may then be supposed to enter the protoplasm of the cell, where it may in turn exist as in the membrane, *i. e.*, physically absorbed in its intermicellary spaces, and from which it may be extracted again by placing the cell in water, as in the first instance. Regarding the manner in which the death of the organism is ultimately brought about, by further increasing the concentration of the dye or the period of time of its action, Stilling assumes that in consequence of the increased storage "the vital movements of the plasmatic micellæ are arrested," death being the outcome. When this has occurred the dye can no longer be exhausted with water. Whether or not chemical union is responsible for this final result he leaves undecided.

Since intracellular metabolism is intimately connected with the action of the enzymes the question has naturally suggested itself whether the deleterious action of the dyes may not in part be referable to interference with the activity of these components. Stilling and Wortmann found as a matter of fact that this may be possible in an indirect way at least, but they have only touched upon this question in a casual way, so that definite conclusions are scarcely warrantable.

On the basis of his experimental studies, which were further extended by animal experiments, in which Stilling could demonstrate that the bacteria in question could actually be killed by the dyes, and that the "pyoctanin" is virtually non-poisonous, he enthusiastically recommended the substance for the treatment of minor surgical infections and advanced many instances to prove its beneficial effect. His therapeutic results were confirmed by several observers,[8] while they were contradicted by others.[9] To judge from the meagre literature one gains the impression as though the subject had been abandoned prematurely, as the laboratory data are certainly encouraging enough to warrant a more laborious investigation of the therapeutic possibilities of the subject.

Following Stilling's publications there are only incidental references to the bactericidal properties of aniline dyes recorded in the literature, the most interesting of which is the paper of Drigalski and Conradi.[10] These writers eliminate the growth of associated acid forming cocci in their method of isolating the typhoid bacillus from the feces, by adding crystal violet, in a dilution of 1 to 100,000 to their medium, as they found that this dye possesses an *elective bactericidal action*, preventing the development of the cocci in question while not interfering with the growth of the typhoid bacillus. Systematic investigations of the selective action of crystal violet or other dyes are not recorded, however.

The first detailed investigations in this direction were made by Churchman, who confined his attention to a single dye, *i. e.*, to gentian violet it is true, but studied the action of this in reference to a large number of pathogenic and non-pathogenic organisms. As a result he divides the various microörganisms into violet positive and violet negative ones, meaning by violet positive that the growth of an organism is inhibited by the violet dye in question, and *vice versa.* The first group he found to include the Bacillus subtilis, Micrococcus aureus and albus, Bacillus diphtheriæ, Bacillus anthracis, Sarcina rosea, Streptothrix actinomyces, Blastomyces, and certain yeasts. The negative group includes the Bacillus prodigiosus, Bacillus pyocyaneus, Bacillus typhosus, Bacillus paratyphosus, Bacillus lactis aërogenes, and the Bacillus suipestifer. This behavior toward gentian violet Churchman found to be "so constant and clear-cut that it must be regarded as a fundamental biological characteristic. A few species have been encountered, like the streptococcus, an occasional strain of which varies in this respect; but for the vast majority of bacterial species and for the vast majority of bacterial strains the behavior toward gentian violet is almost, if not absolutely constant." In comparing the biological behavior of the different organisms toward gentian violet with the behavior of the same ones when studied according to Gram, Churchman was struck with a certain parallelism between the two phenomena. Of the 130 species which were investigated, he found 77 Gram-positive and 53 Gram-negative, and of the former 70 (or 90 per cent.) were violet positive, while of the latter only 8 (or 15 per cent.) were inhibited in their growth by the dye.

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[8] Sée and Moreau, Médecine moderne, July, 1890; Petersen, Petersburger med. Woch., July, 1890; Jänicke, Fortsch. d. Med., June 15, 1890. See also Stilling's second communication.

[9] Garré and Troje, Münch. med. Woch., 1890, No. 25.

[10] Zeitsch. f. Hygiene, 1902, xxxix, 283.

As regards Churchman's technique and many interesting details I must refer the reader to the original. At this place I would, however, emphasize that Churchman was able to distinguish a special strain of the Bacillus enteritidis from four other strains of the same group by its differing behavior toward gentian violet, while in all other respects the five strains were indistinguishable the one from the other.

In reference to the mode of action of the gentian violet, Churchman does not seem to have come to any definite conclusion. He points out that it does not depend on the presence of arsenic nor upon any change in the reaction of the media; presence or absence of light produces no difference, while the question whether or not the effect was due to any radiant properties of the dye could not receive an ultimate answer.

B. PERSONAL INVESTIGATIONS. For some time past I have had in view a systematic study of malignant growth by vital staining, but have thus far not been able to conduct it. Churchman's[11] interesting papers have revived this desire, but in view of the expense of the undertaking it seemed advisable to gain a closer insight into the selective action of aniline dyes upon simpler organisms first, and to study this more specifically from *the standpoint of structural chemistry.*

Technique. The technique which I employed was very simple. Nutrient agar was impregnated with the various dyes in the proportion of 1 to 100,000 and slants then inoculated with the different organisms and incubated for twenty-four hours. At the end of this time the results were noted; a three plus (+++) sign being used to indicate copious growth, double plus (++) fair growth, single plus (+) slight growth, plus over minus (+) almost complete inhibition, and zero (o) absence of any growth. If at the end of twenty-four hours no growth was apparent the tube was returned to the incubator for another period of twenty-four hours; if there was still no growth apparent the surface was gently scraped with the platinum loop and a tube of plain agar, broth, or litmus milk inoculated, according to the nature of the organism. The result was not marked as negative unless the apparent absence of growth on the dye agar was thus controlled and confirmed.

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[11] Churchman, J. W., Jour. exper. méd., 1912, xvi, 221; Churchman, J. W., and Howard, M. V., ibid., 822; Churchman, J. W., ibid., 1913, xvii, 373.

The organisms studied were for the most part the ordinary ones which are pathogenic for man, different strains being procured especially of those which belonged to an apparently dye-positive group; they include the Staphylococcus aureus, Micrococcus catarrhalis, streptococci from different sources, the pneumococcus, the meningococcus, the Bacillus typhosus, paratyphosus *a* and *b*, paracoli and coli, the Bacillus enteritidis, the Bacillus anthracis, the Streptothrix actinomyces, the various strains of the Bacillus dysenteriæ, and the cholera vibrio. In addition some of the more common non- or semipathogenic organisms were studied in reference to certain ones of the dyes such as the Bacillus subtilis, the Micrococcus aurantiacus, Bacillus proteus, Bacillus acid lactis, Bacillus alkaligenes, Bacillus choleræ suum, Bacillus septicæmiæ hæmorrhagicæ, and the Bacillus prodigiosus. The aniline dyes which were investigated include representatives of all the different groups. (See table in second communication.)

The Effect of the Color of the Dye in its Action on Bacteria. From the fact that Churchman divides bacteria into a violet-positive and violet-negative group, and that Stilling comprises all the different violet dyes under the heading of "methyl violet," one gains the impression that these observers attach a certain significance to the violet color of the dye so far as its inhibitory or bactericidal action is concerned. Table I, however, shows that the mere possession of a violet color on the part of an aniline dye does not necessarily carry with it a bactericidal effect, for we see from this that whereas crystal violet, methyl violet B, and dahlia may prevent the development of certain strains of staphylococci and streptococci, gallocyanin, roth violet 5RS, acid violet, and ponceau do not possess this property (in the standard concentration of 1 to 100,000).

Table I.

	Cryst. v.	Methyl v.	Dahlia.	Gallo.	Roth v.	Acid v.	Ponceau.
Micrococcus aureus (1) .	0	0	0	+++	+++	+++	+++
Streptococcus anginæ (78)	0	0	0	+++	+++	+++	—
Streptococcus anginæ (81)	0	0	—	+++	+++	+++	+++

The Effect of the Color of the Dye in Relation to its Acid or Basic Properties. Subsequent studies then showed that the color of the dye may be a factor in the production of the bactericidal effect, but only insofar as the acid or basic character of the substance is concerned. It will be recalled that this is dependent upon the presence of certain auxochromic groups, of which the amino group (NH_2) has basic properties, while the hydroxyl (OH), carboxyl (COOH), sulphoxyl (SO_2OH), nitro (NO_2), and nitroso (NO) groups have acid properties. As is well known the basic dyes are for the most part of right spectral colors, viz., the various shades of violet and blue, while most of the acid dyes are left spectral in character, that is, red, orange, and yellow. Now we find as a matter of fact that those violet and blue dyes which are basic in character may be bactericidal in their action, while the acid red, orange, and yellow dyes do not possess this effect. (See Table II.)

TABLE II.

	Basic right spectral.			Acid left spectral.		
	Cryst. v.	Methyl v.	New methylene blue.	Rosindulin 2G.	Orange G.	Tartrazin (yellow).
Micrococcus aureus (1)	0	0	0	+++	+++	+++
Streptococcus anginæ (78)	0	0	0	+++	+++	+++

Since there are exceptions, however, to the above rule regarding the interrelation between basicity and right spectral colors on the one hand and acidity and left spectral colors on the other, the question naturally suggests itself (1) whether a basic dye of left spectral color may not also possess bactericidal properties, and (2) whether an acid dye of right spectral color may not be capable of similar action.

TABLE III.—Basic Left Spectral Colors.

	Fuchsin.	Phenosafranin.	Tolusafranin.	Asin red.	Rhodamin 3B.
Micrococcus aureus (1)	0	–	–	–	0
Micrococcus aureus (42)	0	–	–	–	0
Streptococcus anginæ (54)	0	–	0	0	–
Streptococcus anginæ (77)	–	0	0	0	–
Streptococcus anginæ (78)	0	0	0	0	–
Streptococcus anginæ (50)	0	–	–	0	0
Bacillus anthracis (14)	–	–	–	0	0

TABLE IV.—Acid Right Spectral Colors.

	Indulin R.	Acid violet.	Gallocy.	Urania blue.	Acid alizarin.
Micrococcus aureus (1)	+++	+++	+++	+++	+++
Streptococcus anginæ (85)	+++	–	–	+++	+++
Streptococcus anginæ (92)	+++	–	–	+++	+++
Streptococcus anginæ (50)	+++	+++	+++	+++	+++
Bacillus anthracis (14)	+++	+++	+++	+++	+++

Table III shows the relation of organisms which readily succumb to basic dyes of right spectral colors when tested against *basic* dyes of left spectral color, while the action of various blue and violet *acid* dyes is shown in Table IV. From the former it is apparent that there are basic red dyes which have bactericidal properties, while Table IV shows clearly that even though the color of a dye be blue or violet it does not follow that such a dye is bactericidal. As a matter of fact, I have not yet met with a dye of either pure or predominatingly acid properties which was bactericidal in character. Some dyes, it is true, have both acid and basic groups, but in the relatively few instances in which bactericidal tendencies were noted in substances of this order the basicity outweighed the acidity.

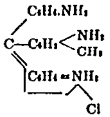
It may then be regarded as an established fact that *an acid dye irrespective of its color* (in the standard concentration of 1 to 100,000 at least) *is devoid of bactericidal properties, while a basic dye, likewise irrespective of its color, may possess inhibitory power.* I regard the elucidation of this fact as important in principle, as it seems to eliminate the possibility that the bactericidal action (in the concentration used) which certain dyes possess could be dependent exclusively upon physical influences. The size of the molecule more particularly could scarcely be an essential factor in the production of the bactericidal effect. In the case of methyl violet 6B (hexamethyl pararosanilin), which is markedly inhibitory, we thus find the molecule composed of 25 atoms of carbon, 30 of hydrogen, 3 of nitrogen, and 1 of chlorine, giving a molecular weight of 397. Amethyst violet, on the other hand, also a basic dye, is made up of 26 atoms of carbon, 31 of hydrogen, 4 of nitrogen, and 1 of chlorine, giving a weight of 414; in other words, it is but little larger than methyl violet and composed of the same elements, and nevertheless its bactericidal power is but very feeble. If, as Stilling suggests, this is essentially due to a closing up of the intermicellary spaces by the large molecules of the dye, then it would be difficult to understand why methyl violet should have a marked bactericidal power, and amethyst violet such of slight degree only, unless indeed it were argued that the latter molecule were too large. The difference between the two is so small, however, as to make this rather unlikely, but even if we were to admit this possibility for argument's sake, it would become invalid in view of the fact that Hoffmann's violet is just as strongly bactericidal as methyl violet, while its molecule is still larger than that of amethyst violet, being a triethyl homorosaniline iodohydrate, $C_{26}H_{32}N_3I$, corresponding to a molecular weight of 493. I have purposely compared methyl violet with amethyst violet, because both are made up of the same elements, and both are basic dyes, as the comparison with an acid dye, containing oxygen or oxygen and sulphur atoms, might be objected to on the basis of the dissimilarity between the two groups in this respect. If, however, this were done it would become even more apparent that the size of the molecule has little if anything to do with the bactericidal effect, as we see it in such weak solutions as 1 to 100,000. While the basicity of the dye is thus an essential factor in determining its bactericidal power it does not follow that all basic dyes possess this property, and the question naturally arises whether there is still another factor in the structure of the dye which is of moment in this respect. When I first considered this question in connection with gentian violet the thought naturally suggested itself that the property might be referable to the underlying triphenyl methane group. A study in this direction showed, as a matter of fact, that it is common to all the red, blue, and violet triamino-triphenylmethanes insofar at least as they are water soluble, and insofar as their basicity is not diminished through the introduction of acid auxochromic radicles.[12] If such radicles are introduced the inhibitory action is lost.

The basic and acid dyes belonging to this group which were studied in this direction, and their structural formulæ, are the following (arranged side by side, so as to show their interrelation):

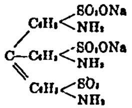
Basic.

Acid.

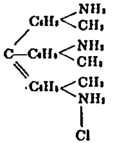
Fuchsin



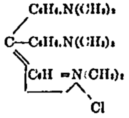
Acid fuchsin



New fuchsin



Methyl violet, 6 B
crystal violet



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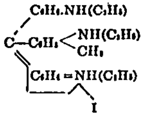
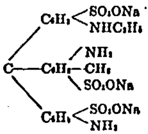
[12] After my own work had been completed and in preparation for the press, Churchman published a third paper on the subject in question, in which he states that he thought it wise to attack the problem by studying substances closely allied to gentian violet in chemical structure, and found that dahlia parafuchsin, magenta, pararosanilin, rosanilin, crystal violet, and methyl violet 5B inhibit the growth of the anthrax bacillus and Micrococcus aureus, while they are of no effect upon the development of the colon and the typhoid bacillus (the only four organisms studied in this direction). He adds that the determination of the chemical group responsible for the bactericidal action of these dyes is the subject of investigations now under way at the laboratory of surgery at Yale University.

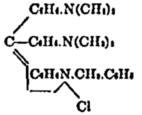
Hofmann's violet



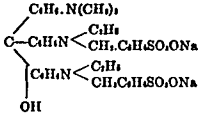
Roth violet
5 R. S.



Benzoyl violet



Acid violet



Dahlia (a mixture of methyl violet and fuchsin.)

The effect of these dyes upon some of the organisms which are of special interest in this question, and the difference in the behavior of the acid as compared with the basic representatives is shown in the following table:

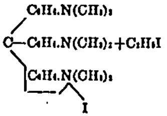
TABLE V.—Showing Inhibitory Effect of Acid Representatives of the Triamino-triphenyl Methane Group (Red, Blue, and Violet Colors).

	Micrococcus aureus (1).	Streptococcus (85).	Micrococcus catarrhalis (6).	Bacillus subtilis (4).	Bacillus anthracis (14).	Actinomyces (15).
Basic:						
Fuchsin	0	0	0	0	0	0
New fuchsin	0	0	0	—	0	0
Methyl violet	0	0	0	0	0	0
Crystal violet	0	0	0	0	0	0
Hofmann's violet	0	0	0	0	0	0
Benzoyl violet	0	0	0	—	0	0
Dahlia	0	0	0	0	0	0
Acid:						
Acid fuchsin	+++	+++	+++	+++	+++	+++
Roth violet	+++	+++	+++	+++	+++	+++
Acid violet	+++	+++	+++	+++	+++	+++

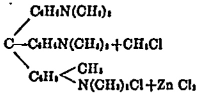
The green basic triamino-triphenyl-methanes are not as uniformly inhibitory as the red, violet, and blue. Of the three which were studied, iodine green is an iodomethylated methyl violet, methyl green, a corresponding chlormethylated product, and ethyl green a bromethylated hexaethyl pararosanilin. All three are triphenyl methanes and all three are basic, but notwithstanding this fact only one, that is, ethyl green, has well-developed inhibitory properties. This difference is no doubt due to the fact that the second dimethylamino group is transformed into an ammonium base by the halogen alkyls, and that the basicity of the compound is thereby lessened.

It may be interesting to point out that here also the introduction of acid auxochromic radicles leads to the disappearance of any inhibitory action that the corresponding basic dyes may possess. The structural formulæ of the dyes in question and the differences in action are shown below (Table VI).

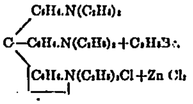
Iodine green.



Methyl green.



Ethyl green.



Echt grün.

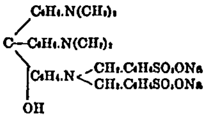
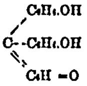


TABLE VI.—Table Showing the Varying Action of the Green Triamino-triphenyl Methanes.

	Basic.			Acid.
	Iodine green.	Methyl green.	Ethyl green.	Echt grün.
Micrococcus aureus (1) . . .	+++	± to +	0	+++
Streptococcus (92)	+++	+++	0	+++
Micrococcus catarrhalis (6) .	+	± to +	+	+++
Bacillus subtilis (4)	+++	0	–	+++
Bacillus anthracis (14) . . .	+++	+++	0	+++
Actinomyces (15)	+++	+	0	+++

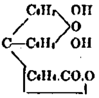
Whether or not some alkyls produce a more marked inhibitory effect than others remains to be seen. Working with uniform concentrations of 1 to 100,000, I cannot say that I could note any essential difference between methylated, ethylated, and benzoylated compounds, unless indeed the relatively more marked inhibitory effect of ethyl green as compared with methyl and iodine green were regarded as evidence of such a difference. The essential factors in any event are not the alkyl groups, but the underlying triphenyl methane group in combination with the three strongly auxochromic amino groups. The importance of the latter has already been illustrated in various ways above, but is further emphasized in Table VII, in which it will be seen that the replacement of the amino by acid hydroxyl or hydroxyl and carboxyl groups leads to a complete suspension of an inhibitory effect. The dyes in question are representatives of the rosolic acid and the phthalein series. Their structural formulæ follow:

Aurin-trioxytriphenyl methane



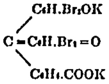
Corallin—constitutional formula not definitely known; probably an intermediary product between aurin and pararosanilin, in which both hydroxyl and amino groups are present.

Fluorescein (dioxy-fluoran)



Uranin—the potassium salt of fluorescein.

Eosin



Erythrosin—the corresponding iodine compound.

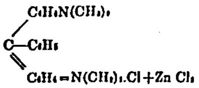
The effect of these dyes upon some of the organisms which ordinarily are inhibited by the triphenyl methane dyes of basic character is here shown.

TABLE VII

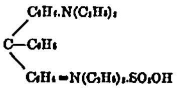
	Corallin.	Uranin.	Eosin.	Erythrosin.	Aurin.
Micrococcus aureus (1) .	+++	+++	+++	+++	+++
Streptococcus (31) . . .	+++	+++	+++	+++	+++
Micrococcus catarrhalis (6)	+++	+++	+++	+++	+++
Bacillus anthracis (14) . .	+++	+++	+++	+++	+++
Actinomyces (15) . . .	+++	+++	+++	+++	+++

The diamino-triphenyl methanes occupy a position which is analogous to that of the green triamino-triphenyl methanes. Like these they are green dyes. I have pointed out that in the latter one amino group has been transformed into an ammonium base, the consequence being that they are virtually diamino compounds. The representatives of this order which were studied are the two basic dyes, malachite green and brilliant green, and the acid Säuregrün S. The formulæ are the following:

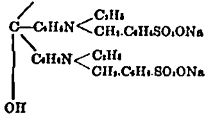
Malachite green.



Brilliant green.



Acid green.



Here also as in the case of the triamino dyes the ethyl compound seems to possess a greater inhibitory effect than the corresponding methyl compound, which very curiously is largely directed against the streptococcus group. (See Table VII.) But the general inhibitory effect is evidently less than in the case of the red, violet, and blue triamino dyes.

TABLE VIII.

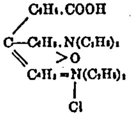
	Basic.		Acid.
	Malachite green.	Brilliant green.	Acid green.
Micrococcus aureus (1)	+++	+++	+++
Streptococcus (50)	0	0	+++
Streptococcus (82)	++	0	+++
Micrococcus catarrhalis (6)	+++	++	+++
Bacillus anthracis (14)	0 to ±	+++	+++
Actinomyces (15)	0 to ±	+	+++

Standing midway between the phthaleins and the trianimo triphenyl methanes are the pyronins, of which rhodamin B and pyronin G were studied. A glance at the formulæ of these bodies will show that they contain two amino groups, and an oxygen atom, replacing one hydrogen atom in two of the phenyl groups, but that rhodamin is a triphenyl product while pyronin contains but two phenyl groups. Rhodamin, moreover, contains a carboxyl group in combination with one of its phenyl groups. As the basicity of one amino group outweighs the acidity of one carboxyl group, we may view rhodamin essentially as a basic dye of the triphenyl methane type, and as such we find that it possesses inhibitory properties, which, however, are not as extensive as those of the triamino products. Pyronin, on the other hand, though markedly basic in character, has no inhibitory action in the concentration which we have chosen as our standard, and the question hence suggests itself whether this may not be owing to the absence of the third phenyl group. The behavior of the single representative of the diphenyl methanes which I have studied, namely, auramin O, suggests that this may indeed be the explanation, though it does not follow that every diphenyl diamino dye should behave in a similar manner. All that we wish to say is that diamino diphenyl products of methane origin do not possess inhibitory properties in the concentration of 1 to 100,000.

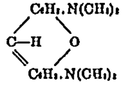
The formulæ of the substances just referred to and their effects upon the organisms in question follow:

TABLE IX.

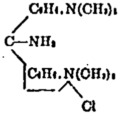
Rhodamin.



Pyronin.



Auramin.



	Rhodamin.	Pyronin.	Auramin.
Micrococcus aureus (1)	0	+++	+++
Streptococcus (92)	+++	+++	+++
Micrococcus catarrhalis (6)	0	+++	+++
Bacillus anthracis (14)	0 to ±	+++	+++
Actinomyces (15)	0 to ±	+++	+++

SUMMARY. 1. The inhibitory effect which certain violet dyes, like gentian violet, methyl violet, dahlia, etc., exercise upon certain bacteria is not referable to their violet color, but to their chemical structure as triamino triphenyl methanes, and is intimately dependent upon their basic auxochromic groups.

2. Acid dyes irrespective of their chemical structure and color, do not possess this inhibitory power.

TYPHLO-ALBUMINURIA.[1]

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IT is not rare to find a clinician assigning an alimentary origin to a case of albuminuria when it cannot be attributed to an existing kidney disease and when the albuminuric phenomenon follows the ingestion of generous amounts of meat, cheese, milk, gelatin, or raw egg albumen. While it is true that in persons with faultless kidneys the ingestion of the raw whites of three or four eggs may result in the excretion of the unchanged ovialbumin, very little, indeed, is known of the albuminurias supposedly occurring in the wake of an overplus of the other protein foodstuffs. In such albuminurias only serins—serum albumin together with serum globulin or serum albumin alone—and no blood-foreign protein are excreted by the urine.

It is for this reason that, apart from ovialbuminuria occasionally following the intake of excessive amounts of raw egg albumen, no definite connection between protein ingestion and the urinary albumin has as yet been established. Every food protein is altered prior to absorption, and though its end-products will ultimately reach the renal secretion, it will not therein reappear in its native physicochemical formation.

There is, moreover, no probability that such a relationship can ever be positively demonstrated so long as we hold to the belief that the protein substance designated by us as serum albumin must have formed a constituent part of the blood. This assumption not only presupposes that the living organism exhibits its contents

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[1] Read at the Annual Meeting of the Medical Society of the State of New York at Rochester, April 30, 1913.